Insulin resistance, renal dysfunction and cardiovascular disease, studies in a high and a low risk population

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CHAPTER 5

Fasting insulin modifies the relation between age and renal function

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Abstract

Background

The worldwide increase in end-stage renal disease has been alleged to insulin resistance-related conditions. Insulin resistance and the concomitant compensatory hyperinsulinaemia may accelerate age-related decline in renal function through inducing glomerular hyperfiltration, even in non-diabetic subjects. This population-based study is the first to investigate whether fasting insulin concentrations modify the relationship between age and renal function in a general non-diabetic population.

Research design and methods

Fasting insulin was measured in 3432 subjects, representing the general population. Cross-sectional analyses of the association between age, renal function and its modification by insulin were performed by means of linear regression. Renal function was assessed as 24h creatinine clearance (CrCl) and 24h urinary albuminuria excretion (UAE).

Results

Age was 48 ± 12 (range: 28-75) years, 44% was male, CrCl was 100 ± 26 ml/min, and UAE was 7.0 [5.4-10.7] mg/24h. The results confirmed a parabolic relation between age and renal function. Fasting insulin modified these parabolic relationships of age with CrCl and UAE ($P<0.001$ for both interaction terms), in such a way that hyperinsulinaemia is associated with a stronger inverse parabolic relation between age and CrCl, and stronger positive parabolic relation between age and UAE at older age than lower insulin concentrations.

Conclusions

Our results are consistent with the hypothesis that insulin accelerates the age-related decline of renal function in the general non-diabetic population. This indicates that insulin resistance, and the concomitant compensatory hyperinsulinaemia could contribute to the increased incidence in end stage renal disease.

Introduction

The incidence of end stage renal disease is increasing worldwide, with the number of dialysis patients growing at an estimated annual rate of 7%.[1] In the Western world, this is mainly attributed to ageing of the population, and to higher prevalence of type 2 diabetes mellitus and renal vascular disease.[2,3] In addition, there is a steady increase in the proportion of subjects in whom no cause for end stage renal disease can be identified.[3] Insulin resistance is alleged to be a common denominator of type 2 diabetes mellitus and vascular disease, and has been argued to underlie many of the unexplained cases of end stage renal disease as well.[4]

Age-associated decline in renal function is thought to result from long-term presence of glomerular hyperfiltration and increased intraglomerular pressure, leading to glomerulosclerosis, albuminuria and finaly nephron loss.[5,6] Despite a diminishing number of functioning nephrons, kidneys can often maintain sufficient renal function through hemodynamic adaptation by hyperfiltration.[7] However, this adaptation may be expected to further increase glomerular pressure, and accelerate the age-related decline in renal function. The notion of an accelerated age-related decline of renal function is supported by population-based studies that show a parabolic relationship between age and renal function.[8]

Insulin resistance is accompanied by compensatory hyperinsulinaemia.[9] High insulin concentrations are known to induce glomerular hyperfiltration.[10] From these experimental data, it has been hypothesized that insulin resistance may contribute to accelerated age-related decline in renal function via compensatory hyperinsulinemia.[11] However, it remains unknown whether compensatory hyperinsulinemia modifies the relation between age and renal function in the general non-diabetic population. Therefore, we investigated the effect of insulin on the relation between age and renal function, as well as urinary albumine excretion (UAE), in a non-diabetic general population.

Methods

Study population and design

The present study population is part of the larger PREVEND study (Prevention of Renal and Vascular End-stage Disease). The protocol of the PREVEND study has been described elsewhere in more detail.[12] From this study, 3432 subjects were selected randomly to obtain a represen-

tative sample of the general population of the city of Groningen, the Netherlands. Non-fasting subjects (n=336), subjects with known renal disease (n=5), subjects with diabetes (n=96) and subjects with missing values (n=42) were excluded from the present analysis, leaving a total of 2953 non-diabetic subjects for analyses. Diabetes was defined as a fasting plasma glucose level of ≥ 7.0 mmol/l and/or use of anti-diabetic drugs or insulin according to the criteria of the American Diabetes Association.[13] Information on drug use was self report, and checked against information from community pharmacies in the city of Groningen as reported previously.[14]

Study subjects completed two visits, which consisted of anthropometric measurements, fasting blood sampling (minimum of 8 hours fasting), ten supine blood pressure measurements taken at one-minute intervals (Dinamap XL Model 9300, Johnson-Johnson Medical, Tampa, FL, USA), gathering of two 24-hour urine collections, and a questionnaire about smoking habits. All participants signed informed consent. The PREVEND study was approved by the Institutional Review Board and was conducted in accordance with the guidelines of the declaration of Helsinki.

Measurements

Systolic and diastolic blood pressures were calculated from the final two recordings at both visits. Body mass index was calculated as weight divided by height squared (kg/m^2). Body surface area was calculated according to DuBois and DuBois.[15] Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Laboratory methods

Plasma and urine creatinine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY, USA). Creatinine clearance was calculated as the mean of two 24-hour urinary creatinine excretions divided by plasma creatinine. Urinary albumin concentration was determined by nephelometry (BNTMII Dade Behring Diagnostic, Marburg, Germany) with a threshold of 2.3 mg/l, and intra-assay and inter-assay coefficients of variation of 2.2 and 2.6% respectively. UAE is given as the mean of two 24h urinary excretions. Sodium and urea urinary excretion were determined on a MEGA clinical chemistry analyzer (Merck, Darmstadt, Germany). Insulin was determined with an AxSym® autoanalyzer (Abbott Diagnostics, Amstelveen, the Netherlands) with a threshold of 7 pmol/l (1.0 µU/ml) and intra-assay and inter-assay coeffi-

cients of variation of 2.6 and 4.3% respectively. This assay shows no cross-reactivity with pro-insulin (0.016% at 106 pg/ml). High sensitive CRP was determined by nephelometry (Dade Behring, Marburg, Germany). Serum concentrations of total cholesterol, HDL cholesterol and triglycerides were measured by standard methods. Glucose was determined in plasma using standard methods.

Statistical analysis

Analyses were performed using SPSS version 12.0 software (SPSS Inc. Chicago IL, USA). Parametric values are presented as mean ± standard deviation, and non-parametric variables as median [inter-quartile range]. Characteristics of the subjects were analyzed over tertiles of fasting insulin. Differences between tertiles were tested with one-way. ANOVA for continuous variables with a normal distribution, Kruskal-Wallis for continuous variables with a skewed distribution, and with Chi-square for nominal variables. A two-sided p-value <0.05 indicated statistical significance

To investigate whether a parabolic association existed between age and renal function in the general population, we first we entered age and age^2 as independent variables in a linear regression model with creatinine clearance as the dependent variable (model 1). Next, we additionally entered insulin, insulin *x* age and insulin *x* age^2 as independent variables to the first model to investigate whether fasting insulin modifies the parabolic relation between age and creatinine clearance (model 2). Significance of the insulin *x* age^2 interaction term indicates that insulin modifies the parabolic relationship between age and renal function.

A similar approach was undertaken with albuminuria as the dependent variable. UAE was logarithmically transformed twice to obtain a normal distribution

To visualise the relation between insulin, age and renal function we stratified our population in tertiles of insulin. In each separate tertile of insulin we entered age and age^2 in linear regression analyses with creatinine clearance as dependent variable. We used the obtained regression formulas to calculate the estimated renal function at every age throughout the age range of our study population for the three separate tertiles. A similar method was used to visualize the relation between age and UAE, again according to tertiles of insulin. In subjects older then 65 years, we analysed whether there was a significant association between insulin and creatinine clearance or UAE using linear regression.

Finally, we performed serveral sensitivity analyses to investigate the

robustness of our findings. In the first sensitivity analysis, we adjusted for gender and adjusted creatinine clearance for body surface area. Secondly, we excluded subjects with a more than 50% difference in urinary creatinine excretion between the two collections, because 24-hour urine collections are prone to collection errors. This method has been used previously.[8] In the third sensitivity analysis, we excluded subjects with impaired fasting glucose (IFG) to exclude potentially under-diagnosed subjects with diabetes. IFG was defined by fasting glucose >5.6 mmol/l 16. Thirdly, we excluded subjects using angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB). This was done because ACEi and ARBs can effect insulin resistance as well as renal function, and therefore confound the associations under investigation.[17] We also performed a multivariate analysis in which we adjusted for gender, BMI, WHR, systolic blood pressure, smoking, fasting glucose, fasting triglycerides, total- and HDL cholesterol and CRP. Finally, we also used Cockroft-Gault and MDRD formulas instead of creatinine clearance as measures of renal function.

All regression coefficients are reported as standardised regression coefficients (unless otherwise specified) to better compare the models amongst each other. Tolerance statistics were used to determine whether assumptions of co-linearity were not violated. Residuals were tested for the normality assumption.

Results

Table 1 shows the characteristics of the study population, according to tertiles of fasting insulin. Fasting insulin increased significantly with age, male gender and all other characteristics shown in table 1. With increasing age, subjects had higher fasting insulin (r=0.14, P<0.001), lower creatinine clearance (r=-0.28, P<0.001) and more UAE (r=0.11, P<0.001).

In model 1 (table 2) we found age to have a parabolic relation with creatinine clearance. This was indicated by the significance of the age^2 term in the regression analysis (age^2: ß= -0.62, P<0.001, R^2=0.09 for the overall model, model 1, table 2). The relation between age and creatinine clearance was modified by fasting insulin concentrations, as indicated by significance of the interaction term of insulin *x* age^2 (ß= -1.66, P<0.001, R^2=0.17 for the overall model, model 2 table 2).

A similar statistical approach was undertaken for UAE as dependent variable (table 2). Age and age^2 showed a parabolic relation with double log-transformed UAE (age^2: ß= 0.67, P<0.001, R^2=0.03 for the overall

Figure 1A

Regression lines of age and creatinine clearance, stratified by tertiles of fasting insulin.

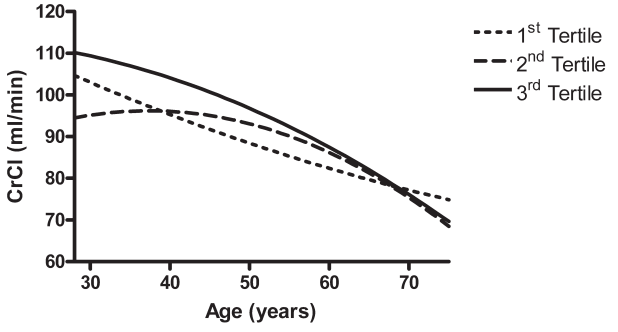
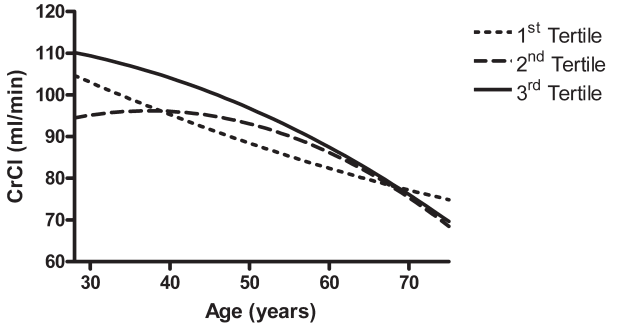


Figure 1B

Regression lines of age and UAE, stratified by tertiles of fasting insulin.



Linear regression analyses of age and age2 as independent variables in each tertile of fasting insulin with either creatinine clearance or UAE as dependent variable. Median insulin (95% CI) concentration for the 1st tertile is 33.0 pmol/l 95%CI (17.2-42.3) (n=975), 2nd tertile 54.5 pmol/l, 95%CI (44.5-66.7) (n=963), 3rd tertile 92.6 pmol/l, 95%CI (70.3- 181.5) (n=964).

model, model 1, table 2). In this model, the interaction term insulin *x* age^2 was also significant (ß= -1.51, P<0.001, R^2=0.04 for the overall model, model 2, table 2).

Figure 1A depicts graphically the linear regression analyses of creatinine clearance and age in each tertile of fasting insulin. The figure indicates that middle-aged subjects with higher fasting insulin concentrations (hyperinsulinemia) have higher creatinine clearance than subjects with lower fasting insulin concentrations. Subjects with higher insulin con-

Table 1. **Population characteristics according to tertiles of fasting insulin.**

n =	975	963	964	Difference
Insulin range, pmol/l	1.4-43.1	43.2-68.2	68.3-218.1	P-value
Age, yrs	47±11	48±12	51±13	<0.001
Male gender, n (%)	411 (42)	415 (43)	460 (48)	0.03
BMI, kg/m^2	23.5±2.8	25.5±3.3	28.4±4.2	<0.001
WHR	0.84±0.09	0.86±0.09	0.90±0.09	<0.001
Systolic blood pressure , mmHg	120±16	125±18	132±18	<0.001
Diastolic blood pressure, mmHg	70±9	73±9	75±9	<0.001
Antihypertensive medication, n (%)	70 (9.3)	118 (15.8)	188 (24.0)	<0.001
of which ACEi/ARB use, n (%)	15 (2.0)	32 (4.3)	53 (6.8)	<0.001
Current smoking, n (%)	348 (35.7)	275 (28.6)	247 (25.6)	<0.001
Total cholesterol, mmol/l	5.3 [4.6-6.1]	5.6 [4.9-6.4]	5.8 [5.0-6.4]	<0.001
HDL cholesterol, mmol/l	1.5 [1.2-1.8]	1.4 [1.1-1.6]	1.2 [1.0-1.4]	<0.001
Triglycerides, mmol/l	0.9 [0.7-1.2]	1.1 [0.8-1.5]	1.5 [1.1-2.1]	<0.001
CRP, mg/l	0.7 [0.3-1.6]	1.1 [0.8-1.5]	1.7 [0.8-3.9]	<0.001
Glucose, mmol/l	4.4±0.6	4.6±0.5	4.9±0.7	<0.001
Insulin, pmol/l	32±8	55±7	106±45	<0.001
Creatinine clearance, ml/min	98±24	100±26	103±28	<0.001
UAE, mg/24h	6.8 [5.3-10.1]	6.9 [5.4-10.3]	7.7 [5.6-11.7]	<0.001

Differences between tertiles were tested with one-way ANOVA for continuous variables with a normal distribution, Kruskal-Wallis for continuous variables with a skewed distribution, and with Chi-square for all nominal variables.

Table 2. **Regression models of age and insulin with creatinine clearance and UAE.**

		Creatinine clearance Standardized ß	P value	UAE Standardized ß	P value
Model 1					
	Age	0.33	0.02	-0.52	0.001
	Age^2	-0.62	<0.001	0.67	<0.001
Model 2					
	Age	-0.54	0.04	-1.35	<0.001
	Age^2	0.26	0.3	1.44	<0.001
	Insulin	-0.87	0.001	-0.99	<0.001
	Insulin x age	2.51	<0.001	2.50	<0.001
	Insulin x age^2	-1.66	<0.001	-1.54	<0.001
Sensitivity analyses					
	Adjustment for gender and BSA[a]	-1.24	0.002	-1.44	<0.001
	Exclusion of urinary collection errors[b]	-1.79	<0.001	-1.64	<0.001
	Exclusion of IFG[c]	-1.66	<0.001	-1.58	<0.001
	Exclusion of ACEi/ARB use[d]	-1.61	<0.001	-1.45	<0.001
	Multivariate[e]	-2.69	0.007	-1.08	0.01

Sensitivity analyses show the coefficients of the insulin x age2 term.

a) Creatinine clearance was adjusted for BSA

b) Subjects excluded with >50% difference in urinary creatinine excretion between the two urine collections (leaving n=2519)

c) Subjects excluded with impaired fasting glucose (leaving n=2701)

d) Subjects excluded using ACEi or ARB treatment (leaving n=2850)

e) Multivariate: adjustment for gender, BMI, WHR, systolic blood pressure, smoking, fasting glucose, fasting triglycerides, total- and HDL cholesterol, and CRP

centrations have a stronger parabolic relation between age and creatinine clearance than subjects with lower insulin concentrations. In subjects older than 65 years, there was no association between fasting insulin and creatinine clearance (ß=0.03, P=0.6).

Figure 1B depicts graphically the linear regression analyses of UAE and age in each tertile of fasting insulin. The figure indicates that UAE is similar at young age for subjects with higher and lower insulin concentrations. With increasing age, however, UAE becomes higher in both groups, but more so in the high insulin group than in the low insulin group. In subjects older than 65 years, there was an association between fasting insulin and UAE (ß=-0.18, P=0.02).

The notion of our findings did not change after adjustment for gender and adjustment of creatinine clearance for body surface area. Further multivariate adjustment for atherosclerotic risk factors did not change the notion of our findings. Exclusion of urinary collection errors, subjects with IFG, or use of ACEi/ARB did not change our findings notably (sensitivity analyses, table 2). Our findings were also not materially changed by using different measures of renal function, namely Cockroft-Gault (insulin *x* age^2 term: P=0.002) or MDRD (insulin *x* age^2 term: P=0.10).

Discussion

The present study is the first to show that fasting insulin concentrations modify the parabolic relation between age and renal function in the general non-diabetic population. The modification indicated that hyperinsulinemia is associated with a stronger parabolic relation of age with creatinine clearance and UAE, than lower insulin concentrations. The present population-based study is therefore consistent with the hypothesis that hyperinsulinemia is associated with an accelerated age-related decline in renal function through induction of glomerular hyperfiltration.[11]

We interpret these findings such that hyperinsulinemia is associated with relative hyperfiltration at younger age leading to accelerated renal function loss at older age. The findings could also be interpreted in such a way that the capacity to hyperfiltrate is lost at older age. However, the concomitant rise in UAE suggests the former: UAE is an accepted marker for renal damage, and higher UAE was already found in young subjects with relative hyperinsulinemia. Furthermore, studies in type-1 and type-2 diabetes mellitus show that hyperfiltration precedes renal function decline and rise in UAE .[18,19] This makes it more plausible that hyperinsulinemia is

indeed associated with accelerated renal function loss rather than loss of existing hyperfiltration.

The relation between fasting insulin and renal function in a population-based study has been only investigated twice previously. In one study the cross-sectional results indicated that fasting insulin was negatively associated with reciprocal of serum creatinine. However, there was no association between insulin concentrations and estimated GFR.[20] In the second cross-sectional study higher insulin concentrations were associated with an increased risk of impaired renal function (estimated GFR below 60 ml/min/1.73m^2). This study also did not take into account a potential acceleration of the age-related decline of renal function [21]. The relation between insulin and the development of microalbuminuria has been studied more thoroughly.[22-25] One prospective study indicated that insulin resistance precedes the appearance of albuminuria in non-diabetic subjects.[24]

There is ample evidence from experimental studies in humans to support that hyperinsulinemia has a detrimental effect on renal function. In humans, the renal and hemodynamic effects of insulin have been studied in detail.[26, 27] In healthy subjects, Stenvinkel et al[26] demonstrated that hyperinsulinemia resulted in time- and dose-dependent increases in renal plasma flow. Likewise, ter Maaten et al[27] found that during insulin infusion, effective renal plasma flow and renal glomerular filtration rate increased with 23.4% (P<0.001) and with 8.8% (P=0.02) respectively. Besides insulin's hemodynamic effects, insulin resistance is associated with adverse changes in numerous cytokines, each of which is thought to contribute to progressive renal function decline as well.[28] These cytokines include plasminogen activator inhibitor-1 (PAI-1), tumor growth factor beta (TGF-ß), and hepatocyte growth factor (HGF).[29-31] Furthermore, insulin also has affinity with the insulin growth factor-1 (IGF-1) receptor, enhancing proliferation of vascular smooth muscle cells.[32]

Furthermore, experimental animal studies support that hyperinsulinemia has a detrimental effect on renal morphology over time. Studies in monkeys show that structural changes, especially glomerular hypertrophy are already present during hyperinsulinemia, before the development of diabetes.[33] Also, obese Zucker rats that were fed ad libitum, developed hyperinsulinemia with renal hypertrophy and glomerulosclerosis.[34] Interestingly, food restriction at early age resulted in lower fasting insulin levels and less kidney disease. Further support for a role of insulin comes from the fact that reduction of insulin and triglycerides concentrations, by treatment with acarbose, resulted in less glomerulosclerosis and reduced

incidence of renal insufficiency in obese Zucker rats.[35]

A limitation of the study is its cross-sectional design, so that cause-efffect relationships can not be inferred. Prospective studies are required to investigate whether hyperinsulinemia is predictive of accelerated deterioration of renal function. Another limitation of our study is that it may have underestimated the true decline in renal function due to healthy survivor bias. Bias may have been introduced because fasting insulin, renal function and UAE are independent predictors of cardiovascular disease morbidity and mortality.[36] Subjects with hyperinsulinaemia, poor renal function, and UAE may have died preferentially compared to other groups before they could enter this study. It should furthermore be noted that declining renal function in association with increased UAE is a relatively late phenomenon. Inclusion bias and this pathophysiologic phenomenon could be why insulin – in the elderly – was not associated with poor renal function, but only with increased UAE.

An important strength of this study is that we used 24h urinary creatinine clearance as measure of renal function and not equations that estimate glomerular filtration rate such as the Cockroft-Gault or the MDRD.[37, 38] This is important because age is included in these equations to correct for lower muscle mass and lower endogenous creatinine production at older age. Because age is included in the formulas for the Cockroft-Gault and the MDRD, their use as independent variables in linear regression analysis, would introduce a statistical fallacy because age would be present in the analysis both as a dependent variable and as an independent variable (incorporated in the estimated GFR), which is statistically not permitted. Therefore, in our opinion, it is methodologically and statistically more correct to use 24h creatinine clearance for this study. The sensitivity analysis indicated that collection errors, which represent the main pitfall in use of 24h urine collections in analyses, did not alter our findings. Furthermore, we also obtained UAE as a marker of renal damage to corroborate our results of creatinine clearance. Other strengths of the study are its large sample size and the availability of pharmacy data. Finally, we used a highly specific insulin assay, ie with very little cross-reactivity with pro-insulin, thereby attributing the current findings solely to insulin and not to pro-insulin or pro-insulin like molecules.

An implication of our findings is that insulin resistance may predispose individuals to accelerated renal function decline even in non-diabetic subjects. Currently there are no diagnostic criteria to allow identification of subjects at risk for progressive renal function decline. One could argue whether this is clinical relevant as the average creatinine clearance of

even the relative hyperinsulinemic elderly in this study was not below 70 ml/min. However, if one already has a diminished renal capacity, insulin resistance could possibly lead to clinically relevant renal function decline even before overt diabetes is present. Insulin sensitizers such as glitazones could be used in insulin resistant individuals to halt hyperfiltration and decrease UAE, thereby protecting against further renal damage, even in absence of diabetes, defined according to present criteria. These implications need to be addressed in future studies.

In conclusion, our results show that fasting insulin concentrations modify the association between age and renal function in the general non-diabetic population. This association indicates that in non-diabetic subjects hyperinsulinemia is associated with an increased age-related decline of renal function and with a concomitant rise in UAE with older age. Prospective studies are needed to give conclusive evidence that higher insulin concentrations indeed accelerate the age-related decline in renal function.

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